Isolated and combined effects of elevated CO_2 and high temperature on the whole-plant biomass and the chemical composition of soybean seeds

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ARTICLE INFO

ABSTRACT

Soybean plants of the variety 'MG/BR Conquista' were grown in open top chambers, simulating elevated CO_2 concentration ($[CO_2]$) and high temperature under the following treatments: 1) ambient $[CO_2]$ and ambient temperature (**Amb**); 2) elevated $[CO_2]$ (eCO_2) and ambient temperature (**Elev**); 3) ambient $[CO_2]$ and high temperature (**Amb/Temp**); 4) elevated CO_2 and high temperature (**Elev/Temp**). The aim was to evaluate responses to elevated $[CO_2]$ and high temperature, with focus on plant development and seed yield, and composition. **Elev** stimulated grain yield and **Amb/Temp** had opposite effect. Several biochemical parameters were affected by **Amb/Temp**, most of them reversed by simultaneous application of **Elev**. The oil obtained with **Elev/Temp** had lower degree of unsaturation. A network of relationships among biochemical parameters of grains at three developmental stages revealed that **Amb/Temp** and **Elev/Temp** affect significantly both carbohydrate and lipid metabolisms. No significant difference was obtained comparing networks corresponding to **Amb** and **Elev/Temp**.

1. Introduction

Over the past few decades, anthropogenic actions have caused a continuous rise in atmospheric CO_2 concentration $[CO_2]$. Recent estimates of $[CO_2]$ increment amounts to 1.5 ppm each year. The global average temperature is expected to rise 2.6–4.8 °C by the end of the century (IPCC, 2014). The effects on agriculture are unpredictable and the performance of crops in a new climate scenario is one of the relevant matters deserving concern (Korres et al., 2016).

Evidence has been accumulated that eCO_2 and high temperature influence food composition (DaMatta, Grandis, Arenque, & Buckeridge, 2010; De Souza, Cocuron, Garcia, Alonso, & Buckeridge, 2015). In the short run, these changes may have implications regarding food security (Challinor et al., 2014).

High contents of protein and unsaturated oil make soybean a major food source in most countries. Estimates have shown that 20% of protein and 25% of lipids of the diet of humans and domestic animals are soybeans-derived (Vara Prasad, Allen Jr, & Boote, 2005).

It has been hypothesized that the expected increase of $[CO_2]$ and temperature in upcoming years might affect the yield, nutritional quality and contents of carbohydrates, protein and lipids of several crops, including soybean (Thomas, Boote, Allen, Gallo-Meagher, & Davis, 2003; Uprety, Sen & Dwived, 2010). Responses of soybean to

climate changes have already been detected in recent years. In India, the rainfall peak has shifted from July to August (Ramkete, Gupta & Singh, 2015). Likewise, in the United States, there has been a shift of temperatures from July to August, as well as a shift from average to intense rainfalls (McFadden & Miranowski, 2016).

Crops respond differently to eCO_2, depending on whether they are C_3 or C_4 photosynthetic systems. Responses of C_3 plants to eCO_2, including soybean, have been intensely studied over the last decades (McFadden & Miranowski, 2016). Using free-air carbon dioxide enrichment (FACE) and open-top chambers (OTC), Bunce (2016) obtained higher soybean yields with eCO_2. Results from SoyFACE studies reveals that eCO_2 increases the rate of photosynthesis, growth rate, grain yield and C/N rate (Bishop, Leakey, & Ainsworth, 2014). On the other hand, high temperature reduces crop plant biomass due to inhibition of carbon assimilation and increases photorespiration rates (Walker, VanLoocke, Bernacchi, & Ort, 2016). Temperatures above 35 °C reduce soybean yield and quality. Data are available also about soybean responses to either eCO_2 or high temperature (Ren, Bilyeu, & Beuselinck, 2009; Khan et al., 2011).

Literature containing evidence of combined effects of both eCO_2 and high temperature on the yield and composition of grains over the development and grain filling is scarce. Baker, Allen, Boote, Jones and Jones (1989) analyzed the development, growth, total nonstructural

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carbohydrate, and seed yield of soybean (cv. Bragg) under eCO_2 and high temperature. Using data obtained only at the end of the harvest, the authors concluded that the responses to eCO_2 are highly dependent on temperature. Although Thomas et al. (2003) observed an effect of eCO_2 on the total nonstructural carbohydrates, oil, and fatty acid concentrations, they did not address the combined effects of eCO_2 and high temperature over the development of soybean grains.

It has been shown that responses of soybean plants to different climate conditions depend on the cultivar being studied. For example, the concentration of oleic acid of soybean grains of cultivars 'Essex', 'Holladay' and 'NK6955' grown under ozone stress is dependent on $[CO_2]$, with highly significant effects depending on the cultivar considered (Uprety et al., 2010). 'MG/BR-46 Conquista' is a variety of soybean introduced by the Brazilian state enterprise Embrapa Soybean Division in 1998 and now widely cultivated in Brazil. This variety is very versatile, covering areas ranging from the southeast to the north and northeast of the country. The objectives of the present study were to evaluate the effects of eCO_2 ($\sim 800\, \mu mol\, mol^{-1}$) and high temperature (4 °C above ambient), separately and in combination, during growth and filling of the grain of soybean and chemical characteristics at three developmental stages and controlled conditions of temperature and CO_2. Besides being the first report on the responses of this widely used soybean variety to the climate change, our study brings the first systemic evaluation of the physiological and biochemical responses of soybean to combined and separate effects of eCO_2 and high temperature.

2. Materials and methods

2.1. Plant material and growth conditions

The treatments to evaluate the effects of elevated $[CO_2]$ and high temperature were carried out in open-top chambers (OTCs) of the laboratory LAFIECO (www.lafieco.com.br), Botany Department, University of São Paulo, Brazil (23° 34' 1" S, 46° 43' 49" W, 783 m above mean sea level) according to Arenque, Grandis, Pocius, de Souza and Buckeridge (2014) with some modifications.

Soybean seeds of the variety 'MG/BR-46 Conquista' were provided by Embrapa Soybean Division (Brasília, DF – Brazil). This variety is well suited to temperatures 20–30 °C. The seeds were germinated at 30 °C with 12/12 h photoperiod in plastic trays with vermiculite. Seven days after germination, 120 seedlings were placed into 5 l pots containing organic soil (mixture of peat, wood *Pinus* cheaps and vermiculite-Plantmax®). At the beginning, each OTC received 30 seedlings. Four polycarbonate OTCs with dimensions of 1.2 m in diameter × 1.8 m in height and visible light (up to 400 nm) at 90% of transmittance were used with the following treatments: 1) ambient $[CO_2]$ and ambient temperature (**Amb**); 2) elevated $[CO_2]$ and ambient temperature (**Elev**); 3) ambient $[CO_2]$ and high temperature (4 °C above ambient) (**Amb/Temp**); 4) elevated $[CO_2]$ and high temperature (**Elev/Temp**). Based on IPCC prognosis, concentrations of ambient $[CO_2]$ and elevated $[CO_2]$ were 380 $\mu mol\, mol^{-1}$ e 800 $\mu mol\, mol^{-1}$, respectively. For **Amb/Temp** and **Elev/Temp** treaments, the temperature inside the chambers was raised 4 °C above ambient with a 1500 Watt resistance, connected to an air flow circulation system and operated by the RICS software (Remote Integrated Control System). Inside the OTCs, elevated $[CO_2]$ was monitored daily with an infrared Testo 535 sensor (Testo Inc., Flanders, NJ, USA); temperature and relative humidity (%) were measured individually every 10 min over the experimental period. All data were recorded with the RICS software (Suplementary 1). Twice a week, 100 mL of Hoagland nutrient solution was added to each pot (Epstein, 1972).

Five plants were randomly selected from each treatment for analysis. Data were obtained from plants in the vegetative and reproductive stages corresponding to 30 (vegetative stage) and 45, 60, 75, 90 and 105 days after emergence (DAE) (reproductive stage). At 30 DAE, the plants had six nodes and no flowers; at 45 and 60 DAE, the plants were in bloom; at 75 and 90 DAE, grains were in growth and filling, and at 105 DAE they reached the maturity stage.

Samples of leaves, stems and roots were dried in a ventilated oven at 70 °C for 72 h. Grains were frozen immediately in liquid N2, lyophilized and powdered with a ball mill.

2.2. Chemical analysis

2.2.1. Carbon, nitrogen and C/N ratio

Carbon and nitrogen contents in powdered grains were determined using a continuous-flow isotope ratio mass spectrometer (IRMS – Thermo-Finnigan Delta PLUS), consisting of an elemental analyzer (MA 1110 – Carlo Erba, Milan, Italy). Carbon and nitrogen ratios were determined to ratio stable isotopes ^{13}C and ^{15}N. Concentration percentages were compared with the standard BBOT (2,5-bis(5-tert-butyl-benzoxazole-2-yl)thiophene), containing 72.703% carbon and 6.522% nitrogen.

2.2.2. Nonstructural carbohydrates

Powdered material from grains (10 mg) was extracted four times with 1.5 mL 80% ethanol for 20 min at 80 °C for analysis of soluble sugars and starch (Arenque et al., 2014). The supernatants were combined and dried in Speed-vac. The pellets were re-suspended wit 1 mL of deionized water. An aliquot of 100 μL was diluted with 400 μL deionized water for quantification of glucose, fructose, *myo*-inositol, raffinose and sucrose by High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD) using a Dionex-DX500 system (Dionex Corporation, Sunnyvale-CA, USA). A CarboPac PA1 column and a 150 mM NaOH solution were used, at a flow rate of 1 mL min^{-1}. Detector responses were determined with solutions of authentic sugar standards.

The residue from the centrifugation was dried and analyzed for quantification of starch, by treatment with thermostable α-amylase and amyloglucosidase. The content of the liberated glucose was determined spectrophotometrically at 490 nm (Amaral, Gaspar, Costa, Aidar & Buckeridge, 2007).

2.2.3. Oil and fatty acids

Oil was obtained from 2 g of powdered material with 300 mL of *n*-hexane in Soxhlet for 6 h. The solvent was eliminated in a rotary evaporator under reduced pressure at 50 °C and the oil maintained in a desiccator until constant weight. Acid-catalyzed transesterification was carried out with methanolic sulfuric acid (Christie, 1993). The fatty acid methyl esters (FAME) were separated from the mixture by treatment with 2% sodium chloride and extraction with dichloromethane. The solvent was evaporated and the FAME dissolved in *n*-hexane. Analysis of FAME distribution was performed by injection of 1 μL of the *n*-hexane solution in a GC/FID system (Agilent HP 5890 Series II Plus chromatograph), equipped with an HP Innowax capillary column (30 m × 0.32 mm × 0.5 μm). The temperature of the injector and detector was 275 °C. Helium was used as carrier gas at 1 mL min^{-1} flow. The column temperature program was 1 min at 150 °C, a first ramp of 15 °C min^{-1} till 225 °C, a second ramp of 5 °C till 260 °C and an isothermal period of 7 min. FAME identification was based on comparison with standards of AOCS rapeseed mix 007 N. FAME relative quantity was evaluated by normalization of the corresponding peak areas.

2.2.4. Protein

Protein content was determined based on total nitrogen content (item 2.2.1). The factor used was 5.50, as recommended by Mariotti, Tomé and Mirand (2008), regarding soybean total protein.

2.3. Statistical analysis

The data corresponding to treatments within the same collection

date were compared by one-way ANOVA and Tukey's test with $p = 0.95$, using JMP® *Statistical Discovery Software* version 5.0.1. Before ANOVA analyses, the null hypothesis of homoscedasticity of variance was tested by four different methods (O'Brien, Brown-Forsythe, Levene and Bartlett).

A correlation network was established to help understanding mechanisms involved in grain maturation, treatment relationships and parameter correlations. Grain biomass and grain contents of oil, soluble protein, nitrogen, carbon, nonstructural carbohydrates (starch, sucrose, fructose, glucose, *myo*-inositol, and raffinose) and fatty acids of grains collected at 75, 90 and 105 DAE were used. Variables in the network are depicted as nodes, while edges (connections) indicate pairwise correlations between component levels. All edges are equally weighed, and Pearson's correlation coefficient between component pairs lies above 0.75. Networks were obtained using the igraph (Csardi & Nepusz, 2006) and R (https://www.r-project.org/) software. The CoGA software (De Siqueira Santos et al., 2015) was used to compare networks obtained with plants grown under four treatments (**Amb**, **Elev**, **Amb/Temp**, and **Elev/Temp**). CoGA identifies structural differences between networks using the degree distribution and the set of degree centrality values. The degree centrality of a node is the total number of edges it stablishes with other nodes. Two networks were considered significantly different if the null hypothesis was rejected, regarding the equality test with an adjusted *p*-value threshold of 0.05.

3. Results and discussion

3.1. Dry biomass of soybean plants

Leaf and stem biomasses increased until 60 DAE with all treatments. Instead, root biomass continued to increase until 105 DAE. The dry biomasses of leaves, stem, and root were affected by treatments and developmental stages (Fig. 1). Up to 60 DAE, **Elev** stimulated increments of leaf biomass. Foliar dry biomass was also influenced by senescence, a process that started around 90 DAE. Senescence speed was increased by **Elev**, **Amb/Temp**, and **Elev/Temp** in such a way that green leaves were completely or almost absent at 105 DAE, as judged by their exiguous biomass (Fig. 1a). Stem and root biomasses were influenced throughout the 105 DAE period by **Elev** and **Amb/Temp**, the former inducing increments and the latter decreasing the biomasses of both organs (Fig. 1b and c). However, the inhibiting effect of **Amb/Temp** and the stimulating of **Elev** is attenuated when both conditions are applied concomitantly (treatment **Elev/Temp**) (Fig. 1a).

The stimulating effect of eCO$_2$ on biomass increment has been attributed to increased photosynthesis rate and carbon assimilation (Rogers et al., 2004; Högy, Keck, Niehaus, Franzaring, & Franzaring, 2010). In contrast, high temperature decreases the biomass of vegetative organs, particularly at the flowering and fruiting stages (Young, Wilen, & Bonham-Smith, 2004). Negative consequences of high temperature have been reported regarding soybean yields (Thomas et al., 2003).

3.2. Number of flowers

The maximum number of flowers was reached at 45 DAE, a period marked by the beginning of pod emergence. Treatment **Elev** increased the number of flowers and anticipated their emergence (Supplementary 2). Treatment **Amb/Temp** apparently retarded the emergence of flowers, but comparing with control (**Amb**), stimulated the formation of flowers, while treatment **Elev/Temp** had no effect on the development and number of flowers (Supplementary 2).

3.3. Grain yield

Fig. 2 shows the increase of grain biomass (Fig. 2a) from 75 to 105 DAE. At full maturity (105 DAE), the number of grains increased with
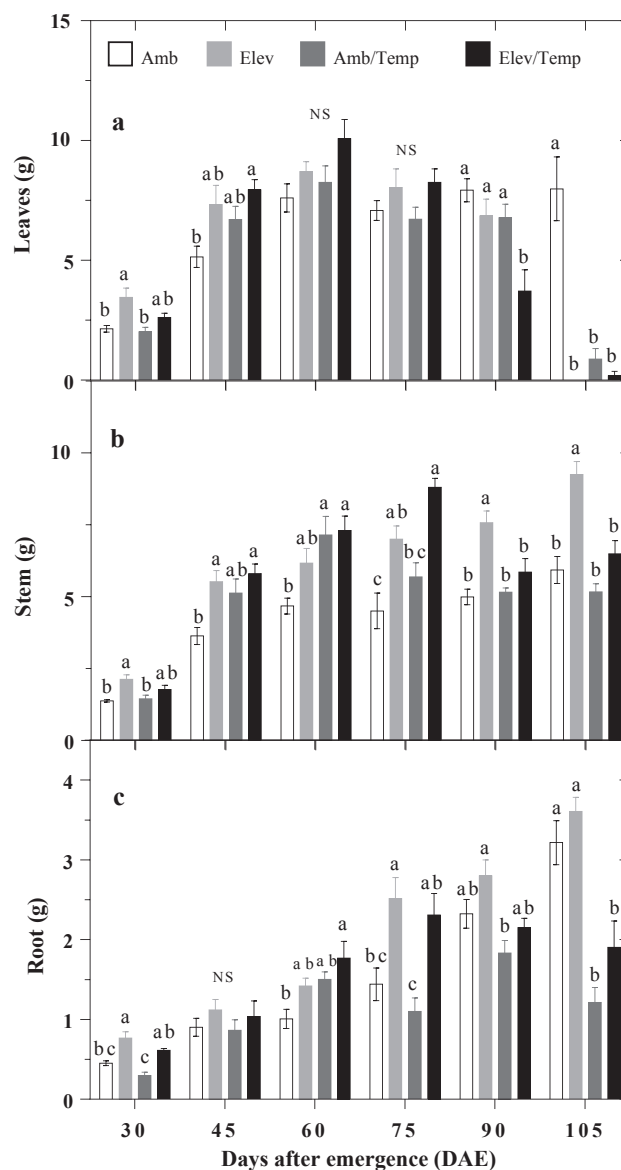

Fig. 1. Effects of treatments **Amb** (ambient [CO$_2$] and ambient temperature), **Elev** (eCO$_2$ and ambient temperature, **Amb/Temp** (ambient [CO$_2$] and high temperature) and **Elev/Temp** (eCO$_2$ and high temperature) on biomasses (g) of leaves (**a**), stem (**b**) and roots (**c**) of plants of the soybean 'MG/BR Conquista' cultivated in open-top chambers. Values followed by the same letters on bars corresponding to the same DAE are not significantly different ($p < 0.05$). NS = Not significant.

Elev (Fig. 2b), in comparison with **Amb/Temp** and **Elev/Temp**, a consequence of anticipated flowering (Supplementary 2). Yields of soybean grains may be improved if simple agronomical practices are adapted to the steadily changing climate conditions. For example, Ramteke et al. (2015) recommend delaying the sowing date of soybean, because of the shift of optimum rainfall period in India. Mourtzinis et al. (2015) highlights the importance of developing local adaptative strategies for climate changes in the United States, where forecasted average yield gains of 30% over the period 1994–2013 were suppressed by changes in precipitation and warming, leading to an estimated loss of US$ 11 billion. Other ways of improving soybean yields are the development of climate-resilient and high yielding varieties, crop rotation, adjustment in cropping calendar and application of farming technological innovation. In developing countries, the adoption of such strategies has still a long way to go but has already achieved increases of soybean productivity of 21.6 kg/ha/year in the Indian Indore district
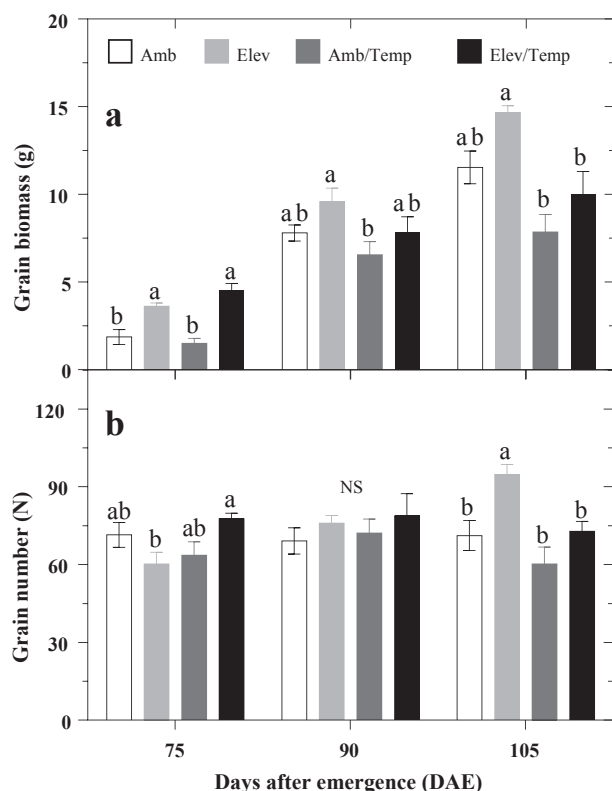
Fig. 2. Effects of treatments **Amb**, **Elev**, **Amb/Temp** and **Elev/Temp** (see caption to Fig. 1) on grain biomass (g) (**a**) and grain number (N) (**b**) of plants of the soybean 'MG/BR Conquista' cultivated in open-top chambers. Values followed by the same letter corresponding to the same DAE are not significantly different ($p < 0.05$). NS = Not significant.

over the period 2001–2013 (Ramkete et al., 2015). Gains of comparable or higher magnitudes are occurring in Brazil, partly derived from the introduction of new soybean varieties, such as 'MG/BR Conquista'. Increase in productivity derived from such strategies is probably more difficult to achieve in developed countries. New modeling methods aiming to interpret yield production, such as the Bayesian dynamic regression, have been introduced in the United States, taking into consideration factors affecting soybean productivity other than climate changes (McFadden & Miranowski, 2016).

3.4. Carbon and nitrogen balance

Both treatments with eCO$_2$ (**Elev** and **Elev/Temp**) affected carbon and nitrogen contents. At 75 DAE, the contents of carbon with both treatments were higher, while the nitrogen contents were lower, in comparison with other treatments (Supplementary 3). During grain development, the differences of carbon and nitrogen contents among treatments were not significant (Supplementary 3). The C/N ratios were lower in grains corresponding to treatments **Elev** and **Elev/Temp** at 75 DAE, but no significant differences were noted at later developmental stages (Supplementary 3).

3.5. Nonstructural carbohydrates in soybean grain

The content of starch in grains gradually decreased with all treatments over the period 75–105 DAE (Fig. 3a). Due to low content in mature grains, starch is rarely addressed in papers about oleaginous seeds. However, starch is a relevant carbon source for the synthesis of many seed metabolites (Gallardo, Thompson & Burstin, 2008). The gradual reduction of starch content along grain development (Fig. 3a) is connected with the mobilization of carbon to the synthesis of soluble

sugars. Likely carbohydrate targets for allocation of starch carbon are sucrose and raffinose. Indeed, the contents of both increased over the whole period (Fig. 3b and f, respectively). The sharpest increase observed was on the raffinose content (Fig. 3f). The variation of fructose and glucose had opposite trends, the former decreasing and the latter increasing over the period 75–90 DAE (Fig. 3c and d, respectively). Treatments with eCO$_2$ (**Elev** and **Elev/Temp**) reduced the content of fructose in grains at 75 DAE. In comparison, **Amb/Temp** did not affect the accumulation of fructose in immature grains (Fig. 3c). In the same period, the glucose content was low with all treatments.

The contents of *myo*-inositol followed a trend similar with starch, i.e., gradual decrease from 75 to 105 DAE, irrespective of treatments (Fig. 3e). The role of *myo*-inositol is crucial for carbohydrate metabolism. It is a precursor to the biosynthesis of galactinol (Sengupta, Mukherjee, Basak, & Majumder, 2015), an essential metabolite for raffinose biosynthesis. Coherently, an opposite trend between the contents of *myo*-inositol and raffinose, the former decreasing and the latter increasing over grain maturity, is evident comparing Fig. 3e and f. The metabolism of *myo*-inositol is sensitive to treatments **Elev** and **Amb/Temp**, the former inhibiting and the latter stimulating its accumulation in grains at 75 DAE and 90 DAE (Fig. 3e). These findings suggest that **Elev** stimulates conversion of *myo*-inositol into other metabolites, for example, raffinose, while **Amb/Temp** has an inhibiting effect during grain maturation. Indeed, it is seen in Fig. 3e and f that the contents of *myo*-inositol and raffinose at 90 DAE fluctuate in opposite directions, comparing **Elev** and **Amb/Temp**. Furthermore, treatment **Elev/Temp** attenuated the effect of **Amb/Temp** (Fig. 3e). In addition to raffinose, other oligosaccharides of the same family accumulate in the process (Thomas et al., 2003).

3.6. Yield of grain oil and distribution of fatty acids

Several authors reported reduction of soybean oil by treatment with elevated temperature (Thomas et al., 2003; Uprety et al., 2010). In the present work, the effect of **Amb/Temp** on grain oil content was dependent on the grain developmental stage. The trends observed for oil contents were inhibition at 75 DAE (although not significant), stimulation at 90 DAE and neutrality at 105 DAE (Fig. 3g). No treatment affected the oil content of fully mature grains (Fig. 3g). Regarding **Elev/Temp**, the results of the present work disagree with Thomas et al. (2003) who reported lower yields of soybean oil under treatment combining eCO$_2$ and high temperature. The explanation for such difference may be related to the varieties used in the two experiments. While a trend of inhibition of oil production in immature grains from plants subject to **Amb/Temp** was detected in grains at 75 DAE, no reduction was observed with treatment **Elev/Temp** (Fig. 3g), similar to previous reports evaluating soybean (cv. 'Thorne') at final harvest Koehler, Huber, Bernacchi, and Baxter (2018).

An important marker of the quality of soybean oil is its fatty acid composition. Our study revealed that the contents of stearic, oleic, and linolenic acids tended to decrease during grain development (Fig. 3i, j and l, respectively). In immature grains, treatments **Amb/Temp** and **Elev/Temp** stimulated accumulation of oleic acid (monounsaturated), but no treatment affected its content in mature grains (Fig. 3j). While the relative proportion of linoleic acid (di-unsaturated) varies little over grain maturity, the content of linolenic acid (tri-unsaturated) decreases (Fig. 3k and l, respectively).

Results shown in Fig. 3l suggest that there is a trend towards decreasing contents of linolenic acid over grain development. Nevertheless, the combination of the treatments of eCO$_2$ and high temperature influenced the content of linoleic acid both at the beginning (75 DAE) and at the final stages of grain filling (105 DAE) (Fig. 3k). The combined effects of eCO$_2$ and temperature (**Elev/Temp**) had a negative influence on the accumulation of linolenic acid in grains at 105 DAE. High temperature alone has no appreciable influence, while **Elev/Temp**-induced the lowest content of linolenic acid in mature grains
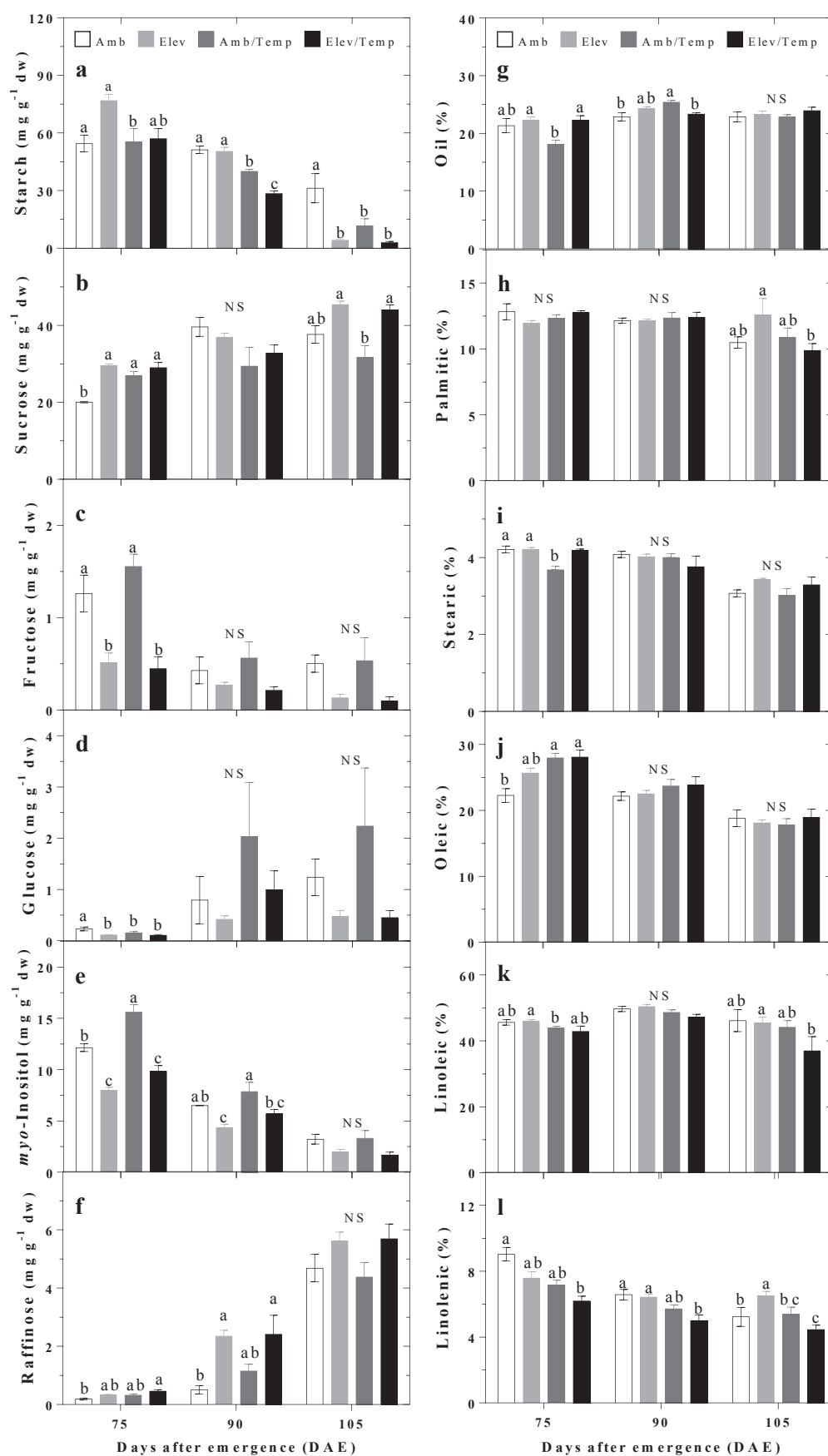
Fig. 3. Contents of starch (**a**) and soluble sugars (mg g^{-1} dw) (**b–f**), seed oil (%) (**g**) and distribution of fatty acids (relative percent) (**h–l**) of mature grains from plants of the soybean 'MG/BR Conquista' cultivated in open top chambers and subject to treatments **Amb**, **Elev**, **Amb/Temp** and **Elev/Temp** (see caption to Fig. 1). Values followed by the same letter corresponding to the same DAE are not significantly different ($p < 0.05$). NS = Not significant.

(Fig. 3l).

According to Uprety et al. (2010), soybean varieties respond differently to climate conditions on various respects, including the distribution of seed oil fatty acids. Linoleic acid is the major fatty acid of soybean oil (Fig. 3k). In our experiment with the variety Conquista, linoleic acid was not influenced by [CO_2] or temperature at stages 75 and 90 DAE (Fig. 3k). However, the **Elev/Temp** treatment reduced the content of linoleic of fully mature grains (Fig. 3k).

Thus, the remarkable change in the combination of fatty acids of mature grains is associated with the content of linolenic acid. Although field experiments will be necessary to confirm these findings, the trend to lower content of linoleic acid (Fig. 3j) suggests the possibility of a decrease of the unsaturation degree of the oil of seeds of the variety 'MG/BR Conquista' as a response to the expected rise of atmospheric CO_2 and temperature in the upcoming years. However, because linolenic acid content of soybean grain is low in comparison with the other fatty acids, no difference is noticed comparing the sum of the percents of saturated and and unsaturated fatty acids (Supplementary 4).

If similar effects are observed in the field, it is expected that the content of total omega acids (unsaturated acids) could remain high. In this case, no appreciable change would be expected regarding the benefits of soybean oil associated with the decrease of the low-density cholesterol and increase of the high-density cholesterol (Sales-Campos, Reis, Santana, & Ribeiro, 2013). However, the possible change in the degree of unsaturation may represent a benefit of the quality of the oil as a biodiesel feedstock, since high degrees of polyunsaturation increase the risk of peroxidation and polymerization of the biodiesel constituents (Islam et al., 2013).

3.7. Proteins

The total protein content of soybean grains gradually increased from 75 to 105 DAE (Fig. 4). Grains collected at 75 DAE from plants subject to **Elev** had lower protein content with treatment **Amb/Temp**, but no change was observed with **Elev/Temp** (Fig. 4). The negative effect of **Amb/Temp** was counteracted by eCO_2. As a result, the total protein content of grains from plants subject to **Elev/Temp** at three developmental stages was not different from the control grains (Fig. 4).

Crop protein concentration responses under eCO_2 have been extensively studied. Metanalysis covering 228 experimental studies involving crops such as potato, barley, rice, wheat, and soybean showed that the protein concentration could be reduced from 15.3% to 1.4% by eCO_2 (Taub, Miller, & Allen, 2008). The underlying mechanisms accounting for this effect are still debatable. It has been hypothesized that

the enrichment of carbohydrates in plant tissues, due to increased photosynthetic rate, and the consequent dilution effect accounts for the decreased nitrogen content (Taub & Wang, 2008).

The effect of high temperature on soybean protein content has been controversial. Khan et al. (2011) reported a decrease in soybean protein by the effect of temperatures within the range 30 and 37 °C. On the contrary, Uprety et al. (2010) reported that high temperature during grain filling increases protein content, while Ren et al. (2009) found no effect of high temperature, although changes on proteomic profiles were noted. It may be speculated that these constrasting results are a consequence of genetic differences among the soybean varieties used in different experiments.

3.8. Correlation network analysis

Fig. 5 shows a comparative analysis of the networks of grains of the soybean 'MG/BR-46 Conquista' under the treatments **Amb**, **Elev**, **Amb/Temp**, and **Elev/Temp**. Networks are formed by nodes (the parameters measured) and links, which represent the Pearson's correlation between variables (straight lines are positive and dashed lines are negative correlations). The number of links in each network can be used to gauge the "cohesiveness" of the network, whereas the number of links that a given node (the degree centrality) receives can be used to evaluate its importance in the network (Barabasi, 2003).

The first trend that can be seen is that the network of **Amb/Temp** presents much higher cohesiveness, followed by **Elev/Temp**, **Elev**, and **Amb**. Some of the nodes with low degrees in **Amb** [such as starch (Sch), fructose (Fru), glucose (Glu), oleic acid (Olc), stearic acid (St) and soluble proteins (SP)] becomes expressive in **Amb/Temp**, **Elev**, and **Elev/Temp** networks.

High temperature (**Amb/Temp**) also increases node degrees. With this treatment, the content of carbon (C), which has a null degree in **Amb** and **Elev**, stands out with the highest degree (8). However, much of the effect of the high temperature is reverted if eCO_2 is concomitantly applied (**Elev/Temp**).

Statistical analysis of the degree centralities revealed that **Amb** differs from **Elev** and from **Amb/Temp**, but not from **Elev/Temp** (Table 1). Therefore, the treatment **Elev/Temp** minimizes the negative effects caused by **Amb/Temp**, in agreement with comments made in previous sections, regarding the reversal of some effects of **Amb/Temp** when **Elev** and **Amb/Temp** were applied in combination. Indeed, **Elev/Temp** treatments have been regarded as a buffering condition for plants (Rosenthal et al., 2014), possibly enabling yield improvements of soybean grains. It is certain, however, that there is an upper limit for high temperatures, above which the damage to grain yields cannot be reverted by eCO_2 (Qaderi & Reid, 2009).

Using network comparisons, our results show that the two separate treatments (**Elev** and **Amb/Temp**) display different whole-plant behavior pathways of development towards the production of seeds. Here we show for the first time that the buffering effect observed when the two treatments are combined (**Elev/Temp**) represents a third and distinct pathway of whole-plant growth and development, which produces seeds that resemble the ones produced by the control plants (**Amb**). This finding highlights the fact that future omics analyses to understand soybean behavior under eCO_2 and high temperature should target the combined treatment to produce more representative results of what may happen in the field, where both variables will change simultaneously with climate changes.

4. Conclusions

The responses of soybean to climate changes, mainly eCO_2 and elevated temperature, depend on the crop variety considered. Previous studies have shown changes regarding yield, plant development, and biochemical features. Several changes were observed in the present work, chiefly if eCO_2 and high temperature (+4 °C) are analyzed
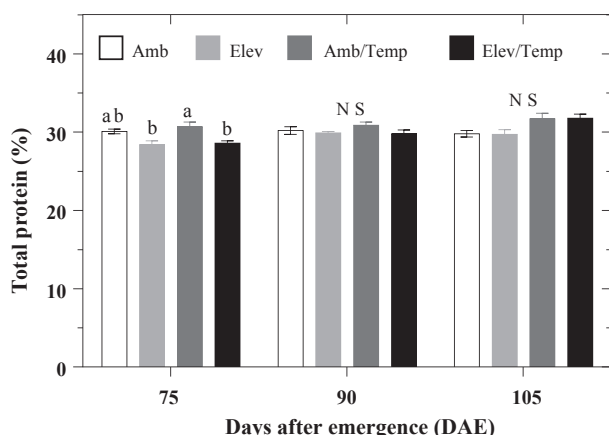
Fig. 4. Contents of total protein (%) of soybean grains from plants of the soybean 'MG/BR Conquista' cultivated in open-top chambers and subject to treatments **Amb**, **Elev**, **Amb/Temp** and **Elev/Temp** (see caption to Fig. 1). Values followed by the same letter corresponding to the same DAE are not significantly different ($p < 0.05$). NS = Not significant.
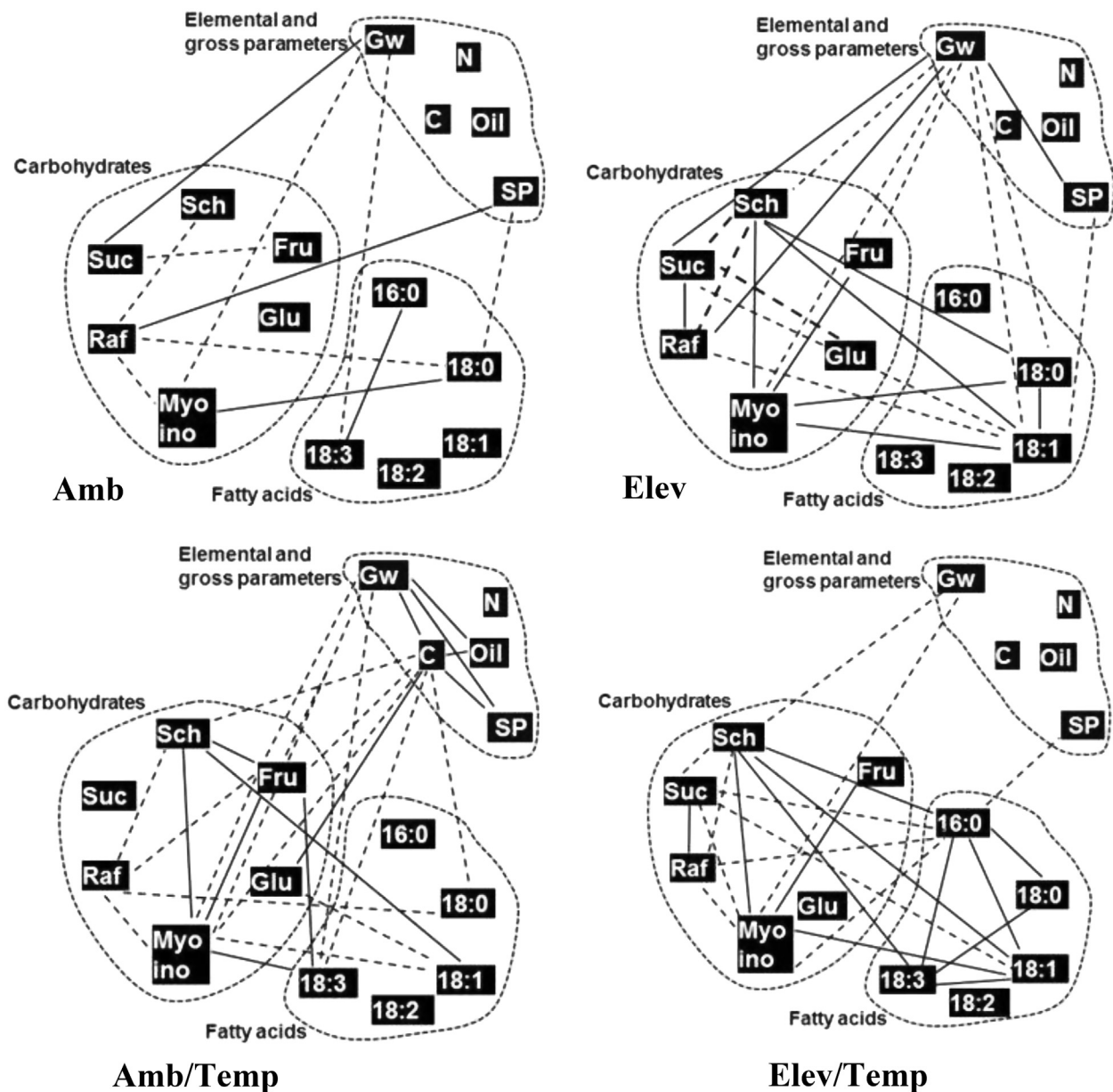
Fig. 5. A network of quantitative relationships among data regarding grains at 75, 90 and 105 DAE from plants of soybean 'MG/BR Conquista', cultivated in open-top chambers and subject to treatments **Amb**, **Elev**, **Amb/Temp** and **Elev/Temp** (see caption to Fig. 1). Gw: grain biomass; C: carbon; N: nitrogen; Oil: content of oil; SP: soluble proteins; Sch: starch; Suc: sucrose; Fru: fructose; Glu: glucose; Myoino: *myo*-inositol; Raf: raffinose; 16:0: palmitic acid; 18:0: stearic acid; 18:1: oleic acid; 18:2: linoleic acid; 18:3: linoleic acid. Solid lines: positive relationships; dotted lines: negative relationships.

Table 1
Significance of differences between degree centralities of correlation networks regarding the effects of treatments **Amb**, **Elev**, **Amb/Temp** and **Elev/Temp** (see caption to Fig. 1) on metabolic parameters of soybean mature grains. *P*-values lower than 0.05 are boldfaced.

	eCO$_2$ (Elev)	High temperature (Amb/Temp)	eCO$_2$ + High temperature (Elev/Temp)
Ambient (Amb)	**0.001**	**0.001**	0.490
eCO$_2$ (Elev)	–	**0.012**	**0.014**
High temperature (Amb/Temp)	–	–	**0.025**

independently. However, when both variables are merged into a combined treatment, most changes are neutralized (a phenomenon named buffering effect), and data regarding plants treated concomitantly with

eCO$_2$ and high temperature did not deviate significantly from results obtained with ambient [CO$_2$] and ambient temperature. Our network analysis revealed that the whole plant behavior of soybean plants under different treatments display distinct pathways of development towards seed production. The combination of eCO$_2$ and high temperature (+4 °C) seems to follow a unique pathway that does not resemble either isolated treatment.

Our results forecast neither reduction or increase in grain yield nor in the oil and protein content of the seeds of 'MG/BR Conquista' plants submitted to an atmosphere containing twice the [CO$_2$] present and a temperature 4 °C above ambient. The comparison between the lower response rates among treatments in the present work with other related studies is possibly associated with a high degree of resilience of the variety used in our experiment. Indeed, the variety 'MG/BR Conquista' is a hardy crop and provides satisfactory yields under a wide diversity of temperature and moisture conditions, from the south to the central

north of Brazil. Even so, our results suggest that the oil obtained from seeds under eCO$_2$ and high temperature (+4 °C) display a slightly lower degree of unsaturation, being consistent with possible changes in food quality and improvement in biodiesel properties in the future. Because this variety is the most widespread in Brazil, which currently produces ca. 30% of the world soybean, our results bring valuable information that could be strategic for future actions towards adaptation to effects of climate change.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.09.052.

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